

CHROMOSOMES OF TWO HYBRIDIZING SPECIES OF *LIMNOPORUS* (HETEROPTERA: GERRIDAE)

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Abstract.—Contrary to an earlier report (Calabrese and Talerico, 1984), *Limnopus notabilis* has a male diploid chromosome number of 21 and an XO male/XX female sex chromosome system. Thus, the cytology of *L. notabilis* is identical to that reported (Calabrese and Talerico, 1982) and confirmed in the present study for a closely related Nearctic species, *L. dissortis*. Male F_1 hybrids between *L. dissortis* and *L. notabilis* from both reciprocal crosses also have a diploid chromosome number of $2n = 20 + X$, and we observed no significant abnormality in meiotic behavior during hybrid spermatogenesis. Because of unusual meiotic behavior, careful study is required to determine the nature of sex chromosome systems in heteropterans. Data about composition of first and second metaphase chromosomes and comparison of chromosome counts from spermatogonial and oogonial mitosis are required to ensure correct interpretation.

Limnopus dissortis (Drake and Harris) and *L. notabilis* (Drake and Hottes) are related Nearctic water-striders that hybridize extensively in nature (Spence, 1981, and in preparation). *L. dissortis* occupies much of North America east of the Rocky Mountains (Brooks and Kelton, 1967), and *L. notabilis* ranges throughout the western U.S.A. and Canada (Polhemus and Chapman, 1979). In Canada, these species occur sympatrically along the eastern slopes of the Rockies and in central British Columbia. The zone of sympatry in central British Columbia is probably the result of a recent expansion of *L. dissortis* (see Scudder, 1977) and extensive introgression between the species is now occurring. Reproductive behavior (Wilcox and Spence, 1986; Spence and Wilcox, 1986) and patterns of interfertility among populations of these two species, as well as gene flow and geographic variation of structural and electrophoretic characters in western Canada, are presently under extensive investigation by one of us (JRS).

Calabrese and Talerico (1982, 1984) reported the chromosome complement of male *L. dissortis* as $2n = 20 + X$, and that of *L. notabilis* as $2n = 18 + XY$. Reports of these chromosomal differences between the two species appeared at odds with the extensive fertility of hybrids of the two taxa and their backcrosses noted in laboratory experiments (Spence, unpublished) and prompted us to reinvestigate the structure and number of chromosomes.

This paper presents observations on the chromosomes of *L. dissortis* and *L.*

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notabilis. In addition, we report on the chromosome complement and meiotic behavior of male F_1 hybrids between *L. dissortis* and *L. notabilis*.

MATERIALS AND METHODS

This paper is based on examination of fourth and fifth instar nymphs of both species and their hybrids. Adults of *L. notabilis*, collected from Marion Lake, near Haney, British Columbia, and those of *L. dissortis* from several small ponds near Swan Hills, Alberta were overwintered in the laboratory at 4°C. These bugs were used to establish laboratory breeding cultures and the resulting offspring were used in this study. Our results are based upon examination of chromosome squashes for 15 nymphs resulting from 7 laboratory crosses (*L. dissortis* and *L. dissortis* F $\times$ *L. notabilis* M, one cross each; *L. notabilis*, two crosses; *L. notabilis* F $\times$ *L. dissortis* M, three crosses). We studied additional squashes of 6 fifth instar nymphs collected from field populations of *L. dissortis* (Dunstable, Alberta) and *L. notabilis* (Fernie, British Columbia).

Gerrid nymphs were fixed in 95–98% ethanol : acetic acid (3:1), with or without prior (1–3 h) injection of 0.01% colchicine in an aqueous solution of 0.9% NaCl, 0.02% KCl, and 0.02% CaCl₂. The testes or ovaries were Feulgen-stained, squashed in 50% acetic acid, and remounted (using the dry ice technique) in Euparal® before observation. Photographs were taken with a Zeiss Photomicroscope I®, with phase contrast optics, using Kodak Panatomic X® film.

Chromosome number was determined for mitotic cells in both male and female preparations and for cells in both meiotic metaphases (MI and MII) in male preparations. When available, 10 well-squashed cells of each stage were counted for each bug; however, if variation in chromosome number was observed among the first 10 counts of any stage, we counted as many cells as possible. In addition, we studied the number of parts contained in each meiotic element. MI elements were either 2-parted or 4-parted, MII elements 1-parted or 2-parted. For all MI and MII cells for which the structure of every chromosomal element could be determined, the numbers of 1-parted, 2-parted, and 4-parted elements were recorded.

RESULTS

As is typical of heteropterans (Ueshima, 1979), *Limnoporus* chromosomes show no evidence of localized centromeres. As well, no m-chromosomes were observed. Sex chromosomes were positively heteropycnotic during meiotic prophase, especially in pachytene (Fig. 1).

Males of both *L. dissortis* and *L. notabilis* have 21 chromosomes (Fig. 2) while females have 22 (Table 1). At comparable stages in the cell cycle, we cannot distinguish between the chromosome complements of the two species. Mitotic chromosomes of both species are small and relatively uniform in size. The only distinctive element in mitotic metaphase is one slightly larger chromosome containing the nucleolar organizer; this chromosome occurs in both species (Fig. 2).

Both *L. dissortis* and *L. notabilis* have a male diploid number of 21 and an XO male/XX female sex chromosome system (Table 1). Female oogonial cells of both species consistently showed 22 chromosomes while mitotic spermatogonia of males showed only 21. In males of both species, meiotic cells in either MI or MII showed 11 elements, only one of which was 2-parted (MI) or 1-parted (MII) (Fig.

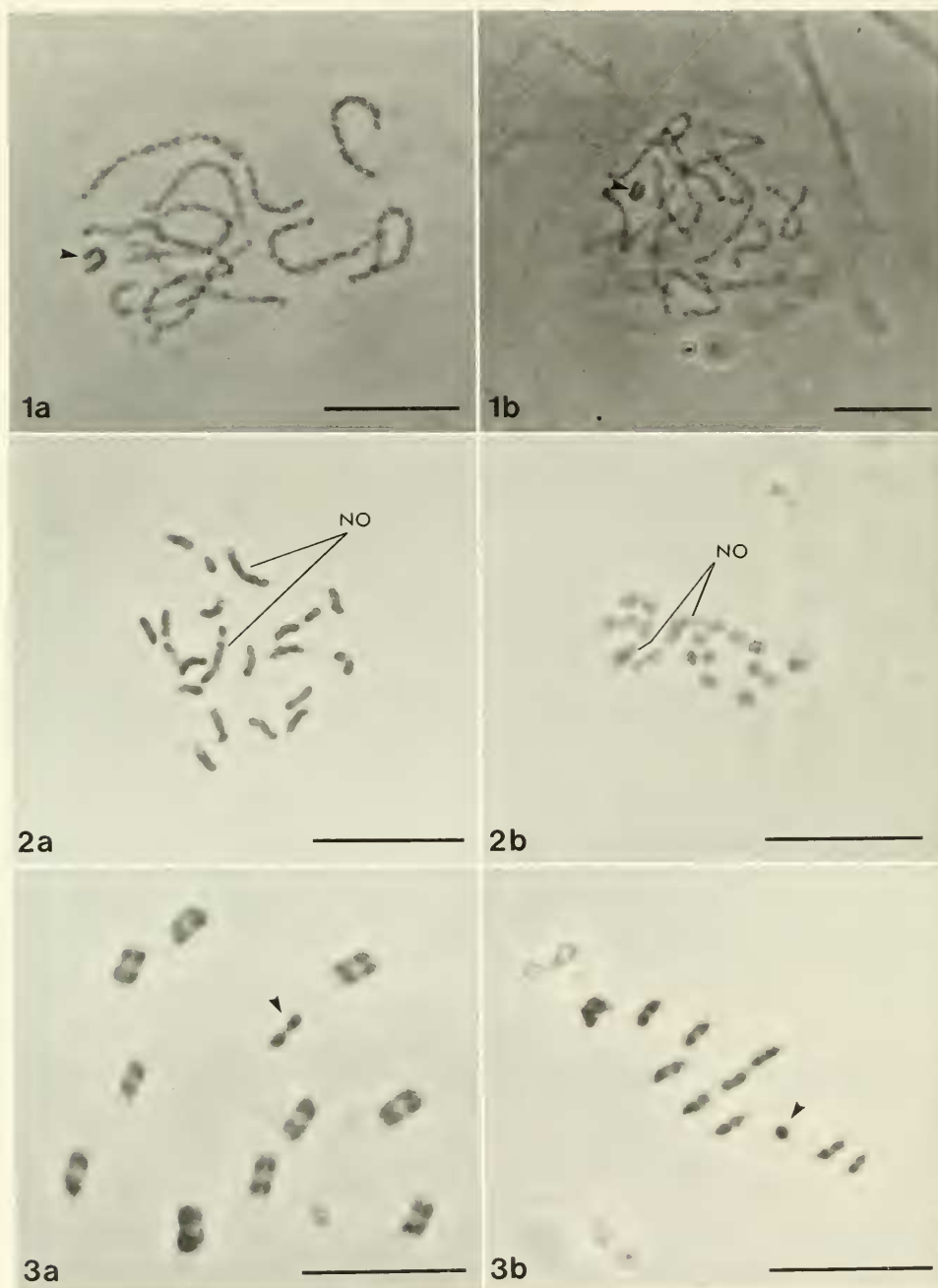


Fig. 1-3. 1, Male pachytene cells showing complete autosomal pairing and the condensed nature of the sex chromosome (X, indicated by arrows) in (a) *L. dissortis* (LIM-3B) and (b) an *F*₁ hybrid (*L. notabilis* male × *L. notabilis* female, LIM-14). 2, Mitotic metaphase chromosomes of (a) male *L. dissortis* (LIM-3B) and (b) male *L. notabilis* (LIM-12). Chromosomes containing the nucleolar organizer indicated by NO. 3, Meiotic chromosomes of male *L. notabilis* (LIM-12), (a) Metaphase I, (b) Metaphase II. Arrows indicate the position of the X chromosomes. Scale bar = 10 μ m.

Table 1. Numbers of cells in which various chromosome counts were observed for specimens of *L. dissortis* and *L. notabilis* and their hybrids. Counts for females are from oogonial mitosis; those for males are from spermatogonial mitosis, and first (MI) and second (MII) metaphase of meiosis. Number of observed cells in which only one chromosome (presumably the X) was clearly 2-parted (MI) or 1-parted (MII) is given in parentheses; no cells with more than one such chromosome were observed, except in hybrids (see text for details).

Species and Sex	Locality	Chromosome Counts								
		Mitosis			MI			MII		
		20	21	22	10	11	12	10	11	12
<i>L. dissortis</i>										
F (LIM-6)	Swan Hills, AB	0	0	12						
M (LIM-5)	Swan Hills, AB	0	10	0	0	7 (6)	0	0	2 (2)	0
M (LIM-3)	Swan Hills, AB	0	10	0						
M (LD-8)	Dunstable, AB				0	10 (10)	0	0	10 (10)	0
M (LD-7)	Dunstable, AB	0	2	0	0	10 (10)	0	0	10 (10)	0
M (LD-5)	Dunstable, AB	0	3	0						
<i>L. notabilis</i>										
F (LIM-2)	Haney, BC	0	1	12						
F (LIM-19)	Haney, BC	0	1	22						
M (LIM-4)	Haney, BC	0	5	0	0	13 (13)	0	0	16 (14)	0
M (LIM-12)	Haney, BC	0	10	0	0	10 (7)	0	0	10 (9)	0
M (LN-41)	Fernie, BC	0	2	0				0	10 (9)	0
M (LN-40)	Fernie, BC	0	3	0	0	10 (10)	0	0	10 (9)	0
M (LN-34)	Fernie, BC				0	10 (10)	0			
Parents: <i>LDF</i> × <i>LNM</i>										
M (LIM-1)	lab reared	3	38	0	0	9 (9)	0	1	17 (16)	0
M (LIM-7)	lab reared	0	3	0						
M (LIM-16)	lab reared	0	10	0	1	28 (26)	0			
M (LIM-17)	lab reared	0	2	0						
Parents: <i>LNF</i> × <i>LDM</i>										
M (LIM-8)	lab reared	1	13	0	0	10 (10)	0			
M (LIM-9)	lab reared	0	10	0				0	3 (3)	0
M (LIM-11)	lab reared	0	2	0						
M (LIM-13)	lab reared	0	11	0	0	17 (16)	4	0	13 (11)	0
M (LIM-14)	lab reared	0	10	0	1	26 (24)	1	0	10 (7)	0

3). Data from hybrid males were also consistent with the hypothesis of an XO male/XX female sex chromosome system in both species (Table 1).

We also studied primary spermatocytes of F₁ hybrids for evidence of abnormal meiotic behavior. Chromosomal pairing during pachytene meiotic prophase seemed to be completely normal for hybrids (cf. Figs. 1a, 1b). In two of nine hybrid males (LIM-13 and LIM-14), a minority of metaphase cells were encountered with 12 distinct chromosomes rather than 11. However, for three of four such MI cells of LIM-13, it was clear that three chromosomes were only 2-parted, suggesting incomplete union of one autosomal pair during meiosis in some primary spermatocytes of hybrids. A few examples of anaphase II, encountered in hybrid slides representing both reciprocal crosses (LIM-1, 2 cells; LIM-13, 3 cells), were apparently normal showing no evidence of chromosomes fragmenting or lagging on the spindle. Overall, it appears that most meiotic behavior in hybrid males is normal and that there is no obvious chromosomal basis for expecting infertility in backcrosses involving these males.

DISCUSSION

Our results indicate that both *L. dissortis* and *L. notabilis* have $2n = 20 + X$ males and $2n = 20 + XX$ females. Thus, our study confirms Calabrese and Talerico's (1982) findings for *L. dissortis*, but disagrees with their (Calabrese and Talerico 1984) report of $2n = 18 + XY$ males in *L. notabilis*. Because Calabrese and Talerico's specimens of *L. notabilis* came from one of the same field populations that we studied, we doubt that the difference in results is explained by within-species variation, but reflects instead an error in interpretation on their part. We outline our reasons below.

Heteropteran chromosomes are unusual in a number of aspects of meiotic behavior. Most important for the present study is the behavior of the sex chromosomes (see White, 1973: 620; Ueshima, 1979: 4-9). In males of most families, if a Y is present, then the X and Y do not form a bivalent at first metaphase. As well, the first division is equational for the sex chromosomes. That is, the X and Y do not migrate to opposite poles (pre-reduction), but instead the two chromatids of the X separate and migrate to opposite poles, as do those of the Y if a Y is present (post-reductional sex chromosomes). Because each sex chromosome splits at first anaphase, each appears rather like an autosomal bivalent at first metaphase. It may thus be difficult to tell if a Y is present or if there is only an additional autosomal bivalent. If a male first metaphase has m "bivalents," then, the specimen may have either $2(m - 2)$ autosomes + XY or $2(m - 1)$ autosomes + X. If positional information is not available (Ueshima, 1979: 4-7), the only clear way to distinguish between these hypotheses from first metaphase cells is by close examination of each "bivalent"; if two of them are clearly 2-parted, and the rest are 4-parted, then the specimen probably has an X and Y; if only one element is 2-parted, then the specimen probably has only an X.

Calabrese and Talerico's published (1982: Fig. 5, 1984: Fig. 5) photographs of first metaphases in the two species are not of sufficient resolution to reveal the structure of each element (cf. Fig. 3). They apparently based their conclusions solely on male first metaphase cells, but they do not state how they determined the sex chromosome system.

To be more certain about the chromosome complement, stages other than first metaphase should be studied. Following is a series of predictions about chromosome numbers and structure for various meiotic stages and mitosis, under two different hypotheses about *L. notabilis*.

Hypothesis 1: sex chromosomes post-reductional, $2n = 18 + XY$; X and Y not forming MI bivalent, and not forming MII pseudobivalent. Predictions: *Males*: 1, MI with 11 elements, 2 2-parted; 2, MII with 11 elements, 2 1-parted; 3, spermatogonial mitosis with 20 elements. *Females*: 4, oogonial mitosis with 20 elements.

Hypothesis 2: sex chromosomes post-reductional, $2n = 20 + X$. Predictions: *Males*: 1, MI with 11 elements, 1 2-parted; 2, MII with 11 elements, 1 1-parted; 3, spermatogonial mitosis with 21 elements. *Females*: 4, oogonial mitosis with 22 elements.

Our results for *L. notabilis* match the predictions for the second hypothesis, $2n = 20 + X$, exactly (Table 1). Note as well that the equal MI and MII counts indicate that the X chromosomes are not pre-reductional.

In some hybrid crosses, nearly 50% of the eggs abort before hatching and F_1 hybrid females from either reciprocal cross are extremely rare or absent (Spence, unpublished). We suggest that rareness of female F_1 's results from interference between X chromosomes originating from females of both *L. dissortis* and *L. notabilis*. However, the more or less normal meiotic behavior observed in hybrid males does not prohibit production of fertile sperm; this conclusion is in accord with results of a study of gene flow between these species using electrophoretic techniques (Spence and Sperling, unpublished).

Although we do not claim that an XO sex chromosome system is necessarily universal among the Gerridae, we hold that present evidence for XY systems is inconclusive. Calabrese and Talerico (1982, 1984) reported presence of Y chromosomes in males of several gerrid species but we find their evidence about *Limnopus* unconvincing for the general reasons provided above. Also, for several additional species, their conclusions are at odds with our preliminary cytological observations. The only other report of Y chromosomes in gerrids known to us is for *G. paladum insularis* by Takenouchi and Muramoto (1968). However, evidence provided by their sketches of spermatocytes is equivocal with respect to the set of predictions that we state above. Their primary evidence is existence of two heteropycnotic bodies in pachytene cells of male meiotic prophase. In this connection, we note that Poisson (1936) reported precocious division of the X chromosome in some individuals of *G. lacustris* and *G. najas*. Such chromosome behavior is also consistent with the description given by Takenouchi and Muramoto (1968), and provides an alternative explanation for the two heteropycnotic bodies illustrated in their Fig. 30. Furthermore, it is not clear from the text that their results are based upon study of more than a single male. The interpretation for *G. paladum* is further clouded because drawings provided by Wilke (1913) suggest an XO system in another subspecies (*G. paladum paladum*). Study of the sex chromosome system was not a specific objective of Wilke's paper and the evidence provided about this point is weak.

To resolve discrepancies like that noted for *G. paladum* above and to promote better understanding of the evolution of chromosome systems in semi-aquatic bugs, future cytogenetic work must be presented with clear statements about how sex chromosome systems were determined. Also, labelled photographs of chromosomes should be provided that are adequate to allow readers to evaluate the interpretations offered.

ACKNOWLEDGMENTS

We thank G. E. Ball and B. S. Heming for comments and discussion that improved this paper, J. S. Scott for preparing the plate, and C. Hiruki and M. Zimmermann, respectively, for assistance with translation of Japanese and German papers. This work was supported by an operating grant to JRS from the Natural Sciences and Engineering Research Council of Canada.

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